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Valproic acid quantification in human serum by LC-MS/MS: method development and comparison with immunoassay

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Valproic acid (VPA) is widely used for epilepsy management, however VPA serum concentration must be monitored because its narrow therapeutic range. Some methods are used to determine the plasma or serum VPA concentration, among them immunoassay-based tests are the most used. Although immunoassays may present cross-responses with other drugs or AE metabolites, increasing the uncertainty of the method. For this reason, a liquid chromatography-mass spectrometry *in tandem* (LC-MS/MS) method was developed, validated and compared with particle-enhanced turbidimetric inhibition immunoassay (PETINIA). For method optimization, the protein precipitation solvent was methanol and isopropanol (3:1) and a biphenyl column (50 x 3.0 mm, 2.7 µm) was used. The method showed selectivity and specificity, and it was linear from 25 to 450 µg/mL, presenting variation coefficient lower than 8,4% and inaccuracy within 10,9%. The matrix effect was 103.9%. The VPA quantification by both methods had good correlation and the VPA assay by LC-MS/MS was 6,85% higher when compared to the immunoassay, but it still gives the same clinical stratification. The validation was successful, with easy sample preparation and appropriate selectivity, applicable for VPA therapeutic drug monitoring.

Keywords: valproic acid; immunoassay; liquid chromatography-mass spectrometry; therapeutic drug monitoring.

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Introduction

The valproic acid (VPA), as seen in Figure 1, is the most used antiepileptic drug (AE) for the management of epilepsy and other neurological disorders (1-2). As others AE, it has a narrow therapeutic range in serum, defined between 50 and 100 µg.mL^{-1}. At concentrations higher than 150 µg.mL^{-1}, VPA presents non-linear pharmacokinetics, resulting from the saturation of protein bindings, making therapeutic control difficult (1, 3-4). Regarding VPA toxicity, overdose is associated with increased penetration into the central nervous system (CNS) and manifestation of neurotoxic symptoms due to CNS depression at concentration up 450 µg.mL^{-1}, such as lethargy or, in severe cases at 850 µg.mL^{-1}, coma. Cases of liver failure, with hyperammonemia, cerebral edema, and metabolic acidosis, are also associated with VPA use (3, 5). For this reason, therapeutic drug monitoring (TDM) is an important strategy to evaluate pharmacotherapy and the best management of the patient during treatment.

Among the methodologies for evaluating the concentration of AE, immunoassays are commonly used for the VPA quantification in serum or plasma because of its low cost. However it has lower sensitivity and selectivity, due to cross-responses with other drugs or AE metabolites, compared to more sophisticated techniques such as chromatographic or mass spectrometry (MS) methods (6-10).

Thus, this paper presents the development and validation of a new liquid chromatography-mass spectrometry *in tandem* (LC-MS/MS) for VPA quantification in human serum. Furthermore, real patient samples in treatment with VPA were tested with the proposed method and the data were compared to the results obtained with the particle-enhanced turbidimetric inhibition immunoassay (PETINIA) to assess the interchangeability between these different methodologies.

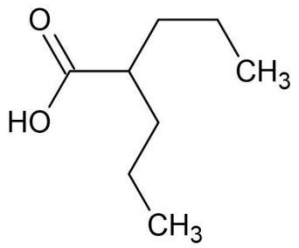


Figure 1 - Structural formula of VPA.

Methodology

The study was previously approved by *Comitê de Ética e Pesquisa da Pró-reitoria de Pesquisa* of the *Universidade Federal do Rio Grande do Sul* (UFRGS), under number 5,913,648.

Equipment and Reagents

The method was developed and validated using a Nexera-i LC-2040C Plus liquid chromatograph coupled to an LC-MS8045 *in tandem* mass spectrometer (Shimadzu, Japan). The raw data were processed using LabSolutions® software (Shimadzu, Japan). Analytical standards and other consumables were obtained from Ceriliant Corporation®, Sigma-Aldrich®, Millipore Corporation®, and Merck®. The Raptor biphenyl chromatographic column (50 x 3.0 mm, 2.7 µm) was obtained from Restek®. The development and validation of the method were processed in the *Central Analític*a of the *Universidade Federal de Ciências da Saúde de Porto Alegre* (CA-UFCSPA).

Method Validation

The validation followed the recommendations of the ICH Guideline M10 on bioanalytical method validation (11), in force from January 2023, covering the parameters: selectivity, specificity, linearity, precision and accuracy, and carry-over, as seen in Table 1. The matrix effect was performed according to Matuszewski *et al.* (2003) (12). The graphical and statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) and Statistica 10.0 software (Statsoft, Tulsa, OK, USA).

Method Comparison

To compare the immunoassay method regularly performed at the *Laboratório de Análises Clínicas e Toxicológicas* (LACT) of the UFRGS with the LC-MS/MS method developed, samples from different patients from LACT were collected and tested. Patient samples were collected from January 2023 to August 2024. Upon receipt at LACT, the samples were dosed at Alinity (Abbott®) using a PETINIA methodology. A small aliquot of the sample, removed at the time of collection, was kept in a freezer at -20°C for evaluation by the method described. Quantitative LC-MS/MS assays were performed in September 2024 at CA-UFCSPA.

For the data obtained by immunoassay and LC-MS/MS statistical analysis, the Passing-Bablok test was used, and the evaluation of the linear relationship was performed by the cumulative sum (CUSUM test), considering a significance of 5%. The comparison data between the methods were also evaluated according to the Bland–Altman plot (13-16). The graphical and statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA).

Clinical stratification, based on the data observed by the two dosage methods, was classified as sub-therapeutic (< 50 $\mu g.mL^{-1}$), therapeutic (50 - 100 $\mu g.mL^{-1}$) and toxic (> 100 $\mu g.mL^{-1}$) and the agreement between the two methodologies was assessed as a percentage (15).

Results and Discussion

Method Optimization and Validation

Sample preparation consisted of aliquoting 100 µL of serum into a 1.5 mL polypropylene microtube, adding 50 µL of 5-(p-methylphenyl)-5-phenylhydatoin (MPPH) as internal standard (IS) at 60 $\mu g.mL^{-1}$, and sequentially precipitating proteins by adding 300 µL of precipitation solvents using methanol and isopropanol (3:1, v/v), followed by centrifugation at 9,000 rpm for 6 minutes. Finally, 1 µL of the supernatant was injected into the analytical system. Figure 2 shows the method flow.

To optimize the solvent used in the first stage, a simplex-centroid multivariate planning was performed based on the absolute areas of the analyte obtained by the method (17). The strategy consists of a series of compositions with different proportions of each solvent, individually prepared and injected into the equipment for analysis in a two-dimensional efficiency scheme, defined by a triangular figure, considering that the three vertices represent each of the organic solvents. Each vertex represents the maximum portion of one solvent and the minimum of the consecutive solvent; in this work, a minimum of zero and a maximum of one (100% of the composition) were stipulated. Furthermore, the points within the triangular figure define distinct combinations of the three solvents, while the sides allow the combination of only two solvents. In total, 10 points were tested to evaluate the best option, as shown in Figure 3, with methanol and isopropanol (3:1, v/v) being adopted as the best option.

To optimize the chromatographic run, it was proposed to use a column with greater retention of polar compounds, such as the Raptor® biphenyl, which has a biphenyl group incorporated into the silica base as a packing. This optimizes the retention of small polar molecules, such as valproic acid, which elute early on C_{18} columns (18). Mobile phase A consisted of 0.2% acetic acid in ultrapure water, while mobile phase B was 0.2% acetic acid in acetonitrile. A gradient method was used as follows: 10% to 100% B in 2 minutes, maintaining 100% B for up to 3 minutes. In 3.01 minutes, 2% B was retained for up to 7.5 minutes. VPA was retained for 2.378 minutes and MPPH for 2.414 minutes. Data acquisition was performed in negative mode. VPA and IS were acquired by Multiple Reaction Monitoring (MRM), using the transitions *m/z* 143 > 143 and *m/z* 265 > 116, respectively.

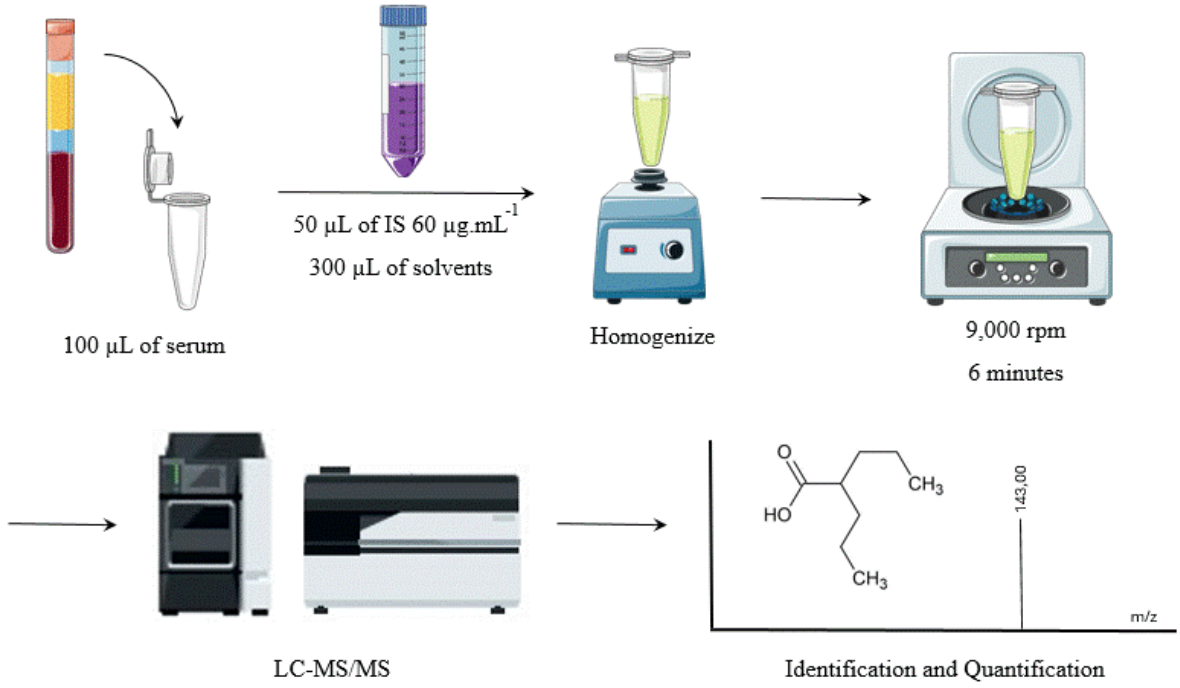


Figure 2 - Proposed VPA quantification method for validation. This figure was generated by editing images provided by Servier Medical Art, provided by Servier and licensed under CC BY 4.0 and illustrations from Shimadzu website.

Table 1 - Validation Parameters

Parameter	Replicates	Acceptance Criteria
Selectivity	6 samples from non-hemolyzed and non-lipemic serum	If interferents are present, these do not present a signal with the same characteristics as the analyte or the IS or, if they present the same characteristics, this interference is not greater than 20% of the LLOQ for the analyte and 5% of the LLOQ for the IS
Specificity	1 sample in triplicate	
Matrix effect	6 samples from different human serum in 4 replicates each at 75 and 360 µg.mL^{-1}	The accuracy in relation to the nominal concentration must be between ±15% and the precision in relation to the coefficient of variation must be equal to or less than 15%
Linearity	6 concentration levels in 6 replicates each at 25 to 450 µg.mL^{-1}	LLOQ must present a variation coefficient equal to or less than 20% of the nominal concentration and the other points equal to or less than 15%. The linear regression should be weighted to reduce the sums of residuals when determined to be heteroscedastic. It is also expected the r^2 greater than 0.99
Precision and accuracy	03 analytical runs with 05 replicates per level in each analytical run at 25, 75, 225 and 360 µg.mL^{-1}	LLOQ must present a variation coefficient and accuracy equal to or less than 20% of the nominal concentration and the other points equal to or less than 15%
Carry-over	1 replicate	In the presence of residual signals, this interference is not greater than 20% of the LLOQ for the analyte and 5% of the LLOQ for the IS

IS, internal standard; LLOQ, lower limit of quantification; r^2, coefficient of determination.

The developed method demonstrated selectivity in all batches of blank matrices tested, with no signals of significant intensity exhibiting the same descriptors (retention time and transition profile) as the analyte under study. The same was observed in the specificity tests, in which the method demonstrated the ability to separate and identify signals from drugs other than VPA, especially other antiepileptics such as carbamazepine, phenytoin, and

phenobarbital. The specificity test also considered the presence of other drugs, none of which interfered with the reading of the analyte under study (Figure 4).

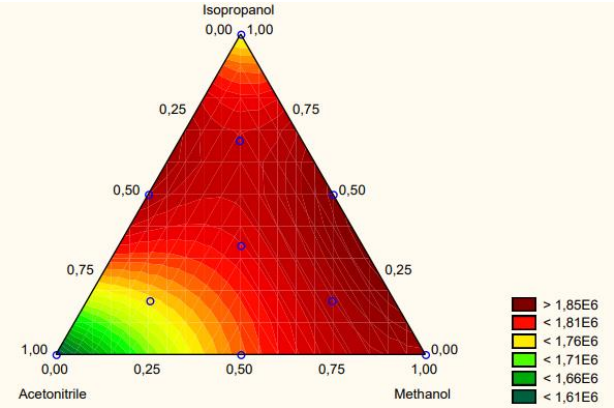


Figure 3 - Simplex-centroid multivariate planning for preparation of precipitation solvent. The graph was created using Statistica 10.0 software.

The linearity range (25-450 μg.mL^{-1}) was determined considering the therapeutic concentration for VPA in serum or plasma adopted in the clinic, being 50-100 μg.mL^{-1} (4); therefore, the lower limit of quantification (LLOQ) was determined experimentally as half the lower concentration of the therapeutic range. After defining the upper level of quantification (ULOQ) as 4.5 times the upper concentration of TDM, a sample of the same level was tested to verify carry-over. No residual signal was observed in the consecutive run after ULOQ injection.

The LLOQ presented a coefficient variation equal to 2.0% and the other points did not present values greater than 5.2%. The linear equation was weighted by $1/y^{1/2}$ and determined as f(Analyte Area/IS Area) = (0.001020 x Concentration) + 0.003391, with the coefficient of determination (r^2) equal to 0.9991.

Precision and accuracy tests (see Table 2) were performed in parallel with linearity analyses, and the same linear relationship obtained was used to quantify VPA in the control samples. For all samples analyzed, LLOQ within-run accuracy results showed inaccuracy within 16.0% and the other points showed no more than 9.1% of the nominal concentration, while inter-run results did not show inaccuracy greater than 8.4%. For precision tests, the coefficients of variation (CV) for both within- and inter-run analyses did not exceed 9.7%, which was the maximum value obtained in a sequence of three runs for LLOQ evaluation. Figure 5 shows a chromatogram obtained from the analysis of a medium-quality control (MQC) sample.

The method showed no significant matrix effect. The analysis revealed the presence of ionization gain in the low-quality control (LQC) samples (111.2%) and suppression in the high-quality control (HQC) samples (96.5%), with the average signal of the samples being 103.9% compared to the analyte in ultrapure water at the same concentration. The coefficient of variation for both LQC and HQC levels was below 15%, averaging 10.6%.

Table 2 - Validation Parameters*

Level	within-run CV (%)	Inter-run CV (%)	Accuracy (%)
LLOQ	8.21	8.43	110.9
LQC	3.03	3.79	106.6
MQC	3.07	3.94	102.5
HQC	4.64	5.08	104.6

* average values obtained during the evaluation of LC-MS/MS proposed method accuracy and precision.

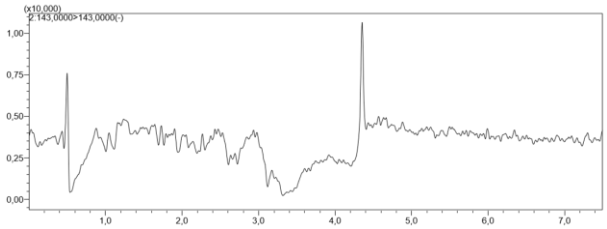


Figure 4 – Chromatogram of specificity test. No signal interferes with the detection and quantification of the analyte.

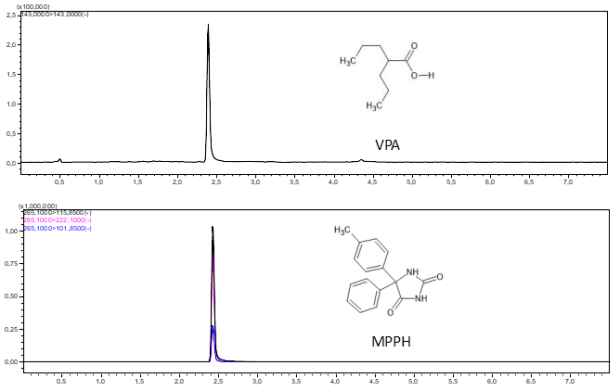


Figure 5 - Chromatogram of MQC sample. Up: VPA identification. Down: MPPH identification, highlighting the *m/z* transitions.

Method Comparison

In total, 22 patient samples were obtained for serum VPA measurement by PETINIA and LC-MS/MS. Of the 22 samples tested, 4 samples (18.2%) showed results below the detection limit for both techniques, respectively 12.5 and 25 μg.mL^{-1} for the PETINIA and LC-MS/MS methods and other 3 samples were below the LLOQ of the LC-MS/MS method. Of the samples subject to quantification, 11 (50.0%) showed a higher value by LC-MS/MS when compared to the results obtained by PETINIA.

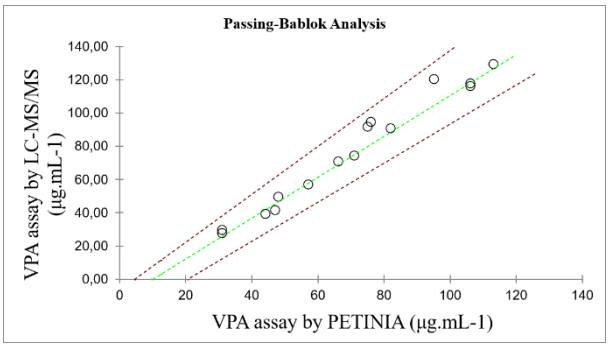
The Passing-Bablok test showed a linear regression defined by LC-MS/MS (μg.mL^{-1}) = 1.2276 x PETINIA

(μg.mL^{-1}) - 12.2470, with a correlation coefficient equal to 0.9862. The CUSUM test did not indicate a significant deviation from the linearity and the 95% confidence interval for the angular coefficient was (1.1757; 1.4410) and for the linear coefficient was (-24.2200; -6.5435). Considering that 1 and 0 are not contained in the intervals of the angular and linear coefficients, respectively, it can be assumed that there is a significant difference between the assay measurements performed by the two methods. The Bland-Altman plot shows a positive bias in the data when quantified by LC-MS/MS: the measurement by this technique results in an average increase of 7.05 μg.mL^{-1} (9.2%), with the absolute deviation becoming more pronounced as the VPA dosage increases. However, when evaluating the percentage distribution of the deviation relative to the mean measurement value by both techniques, the deviation appears to exhibit random behavior. The statistical analysis graphs are presented in Figure 6.

Considering the therapeutic concentration of VPA for analyzing the expected clinical response for the patients tested, only one sample (4.6%) presented a discordant outcome between the two methods: the VPA dosage by PETINIA for the patient was 95 μg.mL^{-1}, while the dosage by LC-MS/MS resulted in 120.4 μg.mL^{-1}. When evaluating this sample, the difference in dosage between the two methods, observed in the Bland-Altman plot, is outside the established range of twice the sample standard deviation. The remaining data were consistent with each other, as shown in Table 3.

In general, most immunoassays overestimate results for the same sample when evaluated by mass spectrometry. The study of Wang *et al.* (2017) presented a comparative VPA analysis between LC-MS/MS and microparticle chemiluminescent immunoassay (CMIA), indicating a good correlation between the two methods, with a positive bias of 1.2 μg.mL^{-1} for CMIA measurement in the range of 2 to 140 μg.mL^{-1} in plasma, using a solid-phase extraction cartridge for sample preparation (16). Another paper, proposed by Zhao *et al.* (2016) proposed to evaluate a method by UPLC-MS against CMIA also obtaining a positive bias for the immunoassay of 0.8 μg.mL^{-1} in the range of 1 to 200 μg.mL^{-1} in serum, preparing the sample through a simple protein precipitation (19).

The method developed in this study presented results that differed from those previously presented, indicating an average increase in the results obtained by LC-MS/MS. However, the results showed good agreement with the therapeutic range defined for VPA. Regarding the number of samples evaluated, this method used a considerably smaller number than Wang *et al.* (2017) and Zhao *et al.* (2016), who worked with 395 and 498 samples, respectively, which is a significant limitation of this study (16, 19). We hypothesized that a small sample size might be a biased representation, not truly reflecting the expected outcome for the population.



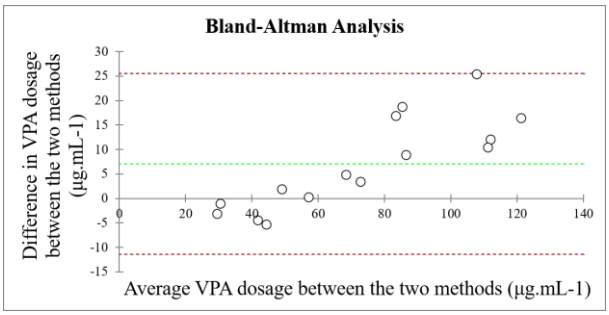


Figure 6 - Up: Passing-bablok plot. Down: Bland-altman plot. The graphs were created using Microsoft Excel.

Table 3 - Comparison between results in relation to the therapeutic range (in μg.mL^{-1})*

Agreement: 95.5%		PETINIA		
		< 50	50 - 100	> 100
LC-MS/MS	< 50	50.0% (11 patients)	0	0
	50 - 100	0	31.8% (7 patients)	0
	> 100	0	4.6% (1 patient)	13.6% (3 patients)

* comparison between VPA dosage data obtained by the two different methods evaluated. Results in agreement for the ranges presented are highlighted in green, and disagreement is highlighted in red. Therapeutic stratification: dosage < 50 μg.mL^{-1} (sub-therapeutic), 50-100 μg.mL^{-1} (therapeutic), and > 100 μg.mL^{-1} (toxic).

Another point to discuss is the VPA stability in the matrix when kept frozen at -20°C. The present method measured samples only in September 2024, after collections had already begun a year and eight months earlier. The developed method did not consider the sample stability study, which may have affected the observed results. However, the work of Zhao *et al.* (2016), which evaluated this parameter considering sample preservation at -20°C for 3 months, did not show significant degradation of VPA (19). Other studies comparing the stability of VPA in the matrix also indicated good stability when stored at -20°C (20, 21), so this variable does not appear to be the

origin of the differences observed between this study and the others mentioned here.

Conclusion

Mass spectrometry is considered the gold standard for quantifying drugs in biological samples. This technique can yield satisfactory results for the VPA analysis in serum and plasma, representing a benchmark method in selectivity and specificity for the analysis of this small molecule, particularly for pharmacotherapy management. The study proposed the development of a simple and rapid method for analyte measurement in serum by LC-MS/MS, using 100 µL of the sample and simple preparation by protein precipitation, obtaining satisfactory results for application in clinical laboratories, respecting the requirements of ICH Guideline M10 on bioanalytical method validation.

When comparing the dosage of serum samples using the method developed by LC-MS/MS in this study, a statistical difference was observed in relation to the same samples assayed by PETINIA, with higher average results for LC-MS/MS, which may be associated with the sampling number. Furthermore, even though studies indicate that valproic acid is stable at -20°C for at least 3 months, the absence of a longer stability study, covering a total period of 8 months, should be carried out to assess the impact of this variable on the observed results. So further studies are necessary to confirm the comparative results between the two methods.

One of the advantages of using chromatographic methods coupled with mass spectrometry is the possibility of using the same method to evaluate several AE. Therefore, there is a future prospect of expanding the number of analytes evaluated by the developed method, especially for the other AE subject to serum level control recommended by the Brazilian Clinical Protocols and Therapeutic Guidelines (PCDT) for epilepsy: carbamazepine, phenytoin, and phenobarbital, establishing regular TDM, enabling better pharmacometrics assessments and improving the pharmacotherapeutic approach.

Author contributions

A.R.S.: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing, L.A.A.: Data curation, Writing – review and editing, J.S.A.: Writing – review and editing, S.C.W.S.E.F.O.: Conceptualization, Methodology, Writing – review and editing, T.F.O.: Conceptualization, Writing – review and editing, B.P.S.: Conceptualization, Data curation, Methodology, Supervision, Writing – review and editing, S.M.C.: Conceptualization, Supervision, Writing – review and editing.

Conflict of interest

The authors declare no conflicts of interest.

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References

1. Freddo RJ. Estudos para obtenção e caracterização de sistemas nanoparticulados contendo ácido valproico e avaliação da penetração deste através da barreira hematoencefálica. Biblioteca Digital de Teses e Dissertações da UFRGS; 2009. Portuguese.
2. Ministério da Saúde (BR). Relação Nacional de Medicamentos Essenciais Rename 2022. Brasília, DF: Ministério da Saúde, 2024. Portuguese.
3. Sztajnkrycer MD. Valproic Acid Toxicity: Overview and Management. J Toxicol. 2002; 40(6):789-801.
4. Patsalos PN, Berry DJ, Bourgeois BFD, Cloyd JC, Glauser TA, Johannessen SI, Leppik IE, Tomson T, Perucca E. Antiepileptic drugsbest practice guidelines for therapeutic drug monitoring: A position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 2008; 49(7):1239-1276.
5. Lheureux PER, Hantson P. Carnitine in the treatment of valproic acid-induced toxicity. Clin Toxicol, 2009; 47(2): 101-111.
6. Eadie MJ. Therapeutic drug monitoring - antiepileptic drugs. Br J Clin Pharmacol. 2001; 52(S1):11-20.
7. McMillin GA, Juenke JEM, Johnson MJ, Dasgupta A. Discordant carbamazepine values between two immunoassays: carbamazepine values determined by ADVIA centaur correlate better with those determined by LC-MS/MS than PETINIA assay. J Clin Lab Anal. 2011; 25(3):212-216.
8. D'Urso A, Ricotta T, de Grazia U. Evaluation of a Novel Immunoassay for Lacosamide Therapeutic Drug Monitoring. Ther Drug Monit. 2017; 39(6):663-668.
9. Jannetto PJ. Therapeutic drug monitoring using mass spectrometry. In: Nair H, Clarke W, editors. Mass spectrometry for the clinical laboratory, 2017. p. 165-179.
10. Strathmann FG, Hoofnagle AN. Current and Future Applications of Mass Spectrometry to the Clinical Laboratory. Am J Clin Pathol. 2011; 136(4):609-616.
11. ICH, EMA. M10 on bioanalytical method validation-Scientific guideline. European Medicines Agency. 2023.
12. Matuszewski BK,Constanzer ML, Chavez-Eng CM. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC–MS/MS. Anal Chem. 2003; 75(13):3019–3030.
13. Hsu SA, Soldo J, Gupta M. Evaluation of two

automated immunoassays for 25-OH vitamin D: Comparison against LC–MS/MS. Journal Steroid Biochem Mol Biol. 2013; 136: 139-145.
14. Giavarina D. Understanding bland altman analysis. Biochem Med. 2015; 25(2): 141-151.
15. Oyaert M, Peersman N, Kieffer D, Deiteren K, Smits A, Allegaert K, Spriet I, Van Eldere J, Verhaegen J, Vermeersch P, Pauwels S. Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact. Clin Chim Acta. 2015; 441: 63-70.
16. Wang Z, Yun Y, Xie X, You C, Miao H, Zhang F, Gao S, Chen W. Comparison of LC-MS/MS vs chemiluminescent microparticle immunoassay in measuring the valproic acid concentration in plasma of epilepsy patients in a new perspective. J Clin Lab Anal. 2017; 32(1):e22157.
17. Bezerra MA, Lemos VA, Novaes CG, de Jesus RM, Filho HRS, Araújo SA, Alves JPS. Application of mixture design in analytical chemistry. Microchem J, 2020; 152: 104336
18. Lupo S, Kahler T, Connolly P. Accurate Pain Management Analysis in Under 5 Min on Raptor Biphenyl Superficially Porous Particle LC Columns. LCGC International. 2015 Mar [cited 2025 Jan]. Available from: https://www.chromatographyonline.com/view/accurate-pain-management-analysis-under-5-min-raptor-biphenyl-superficially-porous-particle-lc-col-2.
19. Zhao M, Li G, Qiu F, Sun Y, Xu Y, Zhao L. Development and Validation of a Simple and Rapid UPLC–MS Assay for Valproic Acid and Its Comparison With Immunoassay and HPLC Methods. Ther Drug Monit. 2016; 38(2):246-252.
20. Xia Y, Long JY, Shen MY, Dong N, Guo HL, Hu YH, Lu XP, Ding XS, Chen F, Qiu JC. Switching between LC-ESI-MS/MS and EMIT methods for routine TDM of valproic acid in pediatric patients with epilepsy: What clinicians and re searchers need to know. Front Pharmacol. 2021; 12: 750744.
21. Yang X, Jiang Z, Jiang Y, Ling J, Dong L, Zou S, Chen R, Hu N. Determination of valproic acid and its six metabolites in human serum by LC–MS/MS and application to interaction with carbapenems in epileptic patients. Biomed Chromatogr. 2022; 37(3), e5572.